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Research Article

Synergistic Activity of Thiadiazole And Thiazolidinone Derivatives Against Alloxan Induced Diabetes In Rats

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Abstract: In our present investigation, different derivatives of 2-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one (3a-3e) were synthesized and evaluated for their antidiabetic activity by using the parameters like serum glucose, serum triglycerides, SGOT, SGPT, and body weight. The compounds were effective in normal values of described parameters. Among these 3a (benzaldehyde derivative) found to be more effective in decreasing the elevated serum glucose level (75.73±8.93 mg/dl) & body weight (214.54±2.75 gms) compared to standard (68.32±3.21 mg/dl) & (131.49±5.09 gms) respectively. The 3d (hydroxyl derivative) was found to be decrease serum triglycerides levels (155.56±13.91mg/dl) & SGOT levels (17.34±2.91) compared to standard (154.38±13.26 mg/dl) & (16.29±2.71) respectively. The 3e (dimethyl amine derivative) found to be decrease SGPT levels (16.13±3.11) when compared to standard (16.29±2.02). The investigation of activity reveled that 3a,3d,3e compounds showed good levels of antidiabetic activity.
Keywords: Diabetic mellitus, SGOT, SGPT, serum triglycerides, serum glucose, alloxan, thiadiazole, thiazolidinone

INTRODUCTION

Diabetes mellitus (DM) is the name given to a multiple group of disorders with different etiologies. It is characterized by derangement in protein, carbohydrate, and fat metabolism caused by the complete or relative insufficiency of insulin secretion and/or insulin action[1]. Diabetes is a major health problem worldwide as approximately 5% of the world's population suffers from diabetes. Worldwide projections suggest that more than 300 million people will have diabetes by the year 2025 and the global cost of treating diabetes and its complication could reach US$1 trillion annually[2]. According to WHO, about 143 million people worldwide suffering from diabeties and the number may likely to double the year 2030[3,4]. Type-2 diabetes was induced in rat models with alloxan monohydrate (40 mg/kg i.v.)[5].

Malihehsafavi[6] etal., explained the importance of synthetic drugs in one review. Thiazolidinone[7,8,9] inhibits the action of Aldose reductase. Compounds carrying the thiazolidinone ring have been reported to demonstrate a wide range of pharmacological activities[10]. Aldose reductase, is an enzyme that is in general found in many parts of the body, and catalyzes the pathway that is responsible for fructose formation from glucose[11]. Katy J brocklehust et al., reported the mechanism of action of two novel and potent direct activators of GK: 6-[(3-isobutoxy-5-isopropoxybenzoyl)amino]nicotinic acid (GKA1) and 5-({3-isopropoxy-5-[2-(3-thienyl) ethoxy] benzoyl } amino)-1,3,4-thiadiazole-2-carboxylic acid(GKA2), which increase the affinity of GK for glucose by 4- and 11-fold, respectively[12].

MATERIALS AND METHOD

Synthesis of derivatives

In synthetic scheme benzoic acid on reaction with thiosemicarbazide yielded 5-phenyl-1,3,4-thiadiazol-2-amine **1** which on reaction with different aromatic aldehydes afforded 5-phenyl-*N*-[(*E*)-phenylmethylidene]-1,3,4-thiadiazol-2-amine derivatives **(2a-2e) .** The compounds **2(a-e)** on treatment with thioglycolic acid in presence of $ZnCl_2$ gave 2-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one **(3a-3e).**

The structures of the compounds were confirmed by melting points, UV-Visible spectroscopy, IR spectra in ^{1}H NMR and MASS spectral data.

The structures of compounds **(3a-3e)** were confirmed on basis of spectral data. IR spectrum showed absorption peaks at 688 cm^{-1} for the C-S-C of

thiadiazole and absorption peaks around 1600cm^{-1} and 2900 cm^{-1} for the C=O & NCH_2S of thiazolidinone respectively. The ^{1}HNMR spectra exhibiting multiple peaks attributed to the protan at δ 7.2- 8 indicating the presence of aromatic protan while siglets at δ 2.5(CH_2), δ 5.9 (CH) indicating the thiazolidinone linkage.

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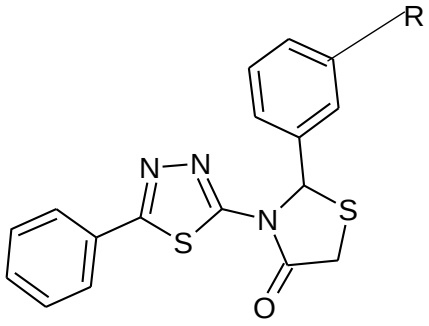
Derivative	Functional group(R)
3a	H
3b	2-Cl
3c	3-NO_2
3d	3-OH
3e	N(CH_3)$_2$



Synthetic drugs are synthesised in chemistry laboratory

Experimental animals

Male Wister albino rats weighing 150-200 g were used in the present study. They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature and relative humidity. Animals were given standard rat pellets (Pranav Argo's ltd.) and drinking water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of Creative Educational Society College of Pharmacy (1305/ac/09/CPCSEA).

Chemicals and Reagents:

Normal Saline, Alloxan, Glucose kit, Triglyceride kit , SGOT kit, SGPT kit.

Equipments: Auto analyzer (MISPAL).

Acute Toxicity Studies :

Experimental Procedure :

The animals were randomly selected , marketed to permit individual identification,& kept in their cages for atleast 5 days prior to dosing to allow for acclimatization to the laboratory conditions. Animals were fasted over night with water *ad libitum* & food was withheld for 3-4 hrs after oral administration of the drug. The principles if laboratory animal care were followed. The animals were divided into 8 groups the test substance was administrated in a single dose by gavage using a suitable intubation cannula.

Anti-diabetic Study:

Drugs Used:

Glimepiride was given to rats at a dose of 40 mg/kg body weight, as a reference standard.

Induction Of Diabetes:

In the present study, diabetes was induced by single intraperitoneal injection of alloxan (40mg/kg) . The alloxan was freshly prepared by dissolving 40mg of alloxan in 10ml of normal saline solution. The animals were allowed to drink 5% glucose solution overnight to overcome the drug induced hypoglycemia.

Groups Design:

Rats were fasted overnight and divided into five groups with 6 animals in each group. Group-8 received distilled water, to serve as control. Group-6 animals were treated with glimepride (200 mg / kg) to serve as standard. Group-7 was treated as diabetic control , while other groups were treated with test compounds. Treatment with compounds was started on 3rd day of alloxan treatment. All drugs were given orally as a single dose.

Table-1: group design

GROUP	TREATMENT
1	3a
2	3b
3	3c
4	3d
5	3e
6	Standard
7	Diabetic control
8	Control

Collection of blood sample

The blood samples were drawn on 7th, 14th and 21st day from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after a fast of 12 hrs and the blood was centrifuged (2,500 rpm/10min) to get serum. The serum was used for biochemical estimation of blood glucose, triglycerides, SGOT,SGPT.

Biochemical Estimations (Serum Analytical Methods)

serum glucose**,** triglyceride(TG)**,** SGOT, SGPT kit were obtained from span Diagnostics Ltd., Surat, Gujrat. Blood glucose, TG, SGOT, SGPT levels in serum were measure using a semi-autoanalyzer as per methods described by the manufacturer.

Statistical Analysis:

All the values were expressed as mean ± Standard error mean (SEM). The differences were compared using one-way analysis of variance (ANOVA) followed by Dunnet's t test. *P* values <0.01 were considered as significant.

RESULTS

Acute Toxicity Studies

Animals were observed individually after dosing at least once during the first 30 mins, periodically during the first 24hrs, with special attention given during the first 4 hrs & daily there after, for a total of 14 days. The time at which signs to toxicity appear & disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations were systemically recorded with individual records were maintained for each animal. The attention was directed to observations. At the end of study the toxicological effect was assessed on the basis of mortality noted after 24hrs. the observed parameters after the test drugs administered are tabulated.

Animals exhibited a significant toxic symptoms during the treatment period in groups given 200 mg/kg of different test drugs orally. Th showed changes in general behavior at the dose level of 200 mg/kg. Hence it can be concluded that a test substance is practically having mild toxicity after an acute exposure at the dose range of 200 mg/kg. From this dose $1\backslash10^{th}$ of the dose 20 mg/kg was selected for the further pharmacological evaluation.

Table -2: Acute Toxicity Studies

Compound	Dose (mg/kg)					
	Groups					
	I	II	III	IV	V	VI
3a	5	10	20	40	80	200
3b	5	10	20	40	80	200
3c	5	10	20	40	80	200
3d	5	10	20	40	80	200
3e	5	10	20	40	80	200

Table-3: Drugs acting on blood glucose levels

Group	Glucose (mg/ml)		
	0 Day	**7^{th} Day**	**14^{th} Day**
3a	254.15±15.04*	102.81±9.15	75.73±8.93***
3b	273.46±14.7	107.87±11.5	92.19±5.93*
3c	260.2±1.34*	131.62±8.56	93.67±6.83*
3d	263.20±3.59	116.62±9.53	82.15±6.13**
3e	273.10±17.04	120.34±10.42	90.58±7.09*
Std	272.28±7.02*	99.72±9.52*	68.32±3.21
D.C	267.21±17.11*	268.45±13.85	259.86±13.69
Control	63.2±1.61	61.31±1.16	63.29±1.34

Values are expressed as mean±S.E.M.,***P<0.001 when compared with diabetic control, **P<0.01 when compared with diabetic control ; *P<0.05 when compared with diabetic control.

Table -4: Drugs acting on serum triglyseroids levels

Group	Triglyseroids(mg/ml)		
	0 Day	**7^{th} Day**	**14^{th} Day**
3a	220.15±15.04*	179.52±14.68	173.32±13.67
3b	221.46±14.7*	191.65±13.52	164.19±14.09
3c	219.2±1.34*	182.72±11.42	157.72±14.23*
3d	218.20±3.59	183.18±14.81	155.56±13.91*
3e	223.10±17.04*	190.16±11.74	163.12±11.12
Standard	212.78±13.08*	174.63±13.42*	154.38±13.26
Diabetic control	220.13±16.11	258.41±14.35	269.09±12.71
Control	143.67±12.56	151.78±13.02	149.23±16.13

Values are expressed as mean±S.E.M.,***P<0.001 when compared with diabetic control; **P<0.01 when compared with diabetic control; *P<0.05 when compared with diabetic control.

Table-5: Drugs acting on serum SGOT levels

Group	SGOT (units per litre of serum)		
	0 day	**7th day**	**14th day**
3a	28.14±3.85	21.34±3.32	18.24±3.05*
3b	27.56±3.83	19.43±3.24	18.57±2.87*
3c	26.14±3.75	20.36±3.09	19.81±3.70
3d	26.75±3.87	19.56±3.05	17.34±2.91*
3e	27.34±3.85	20.02±3.12	18.16±3.09*
Standard	27.04±3.47	19.67±3.72	16.29±2.71
Diabetic control	26.89±3.63	29.15±4.12	32.72±4.41
Control	15.16±3.71	15.43±3.83	14.56±3.73

Values are expressed as mean±S.E.M., ***P<0.001 when compared with diabetic control; **P<0.01 when compared with diabetic control; *P<0.05 when compared with diabetic control.

Table -6: Drugs acting on serum SGPT levels

Group	SGPT(units per litre of serum)		
	0 day	**7th day**	**14th day**
3a	27.09±3.92	20.34±3.02	18.06±3.32
3b	28.13±3.16	19.03±2.67	18.42±3.64
3c	28.52±3.32	21.84±3.73	17.06±2.73*
3d	27.02±3.18	20.73±3.28	18.69±2.86
3e	28.92±3.64	22.62±3.73	16.13±3.11**
Standard	26.72±3.02	18.61±2.72	16.29±2.02
Diabetic control	28.25±3.08	30.23±3.95	33.43±3.99
Control	14.31±2.53	13.61±2.64	13.32±2.40

Values are expressed as mean±S.E.M.,***P<0.001 when compared with diabetic control; **P<0.01 when compared with diabetic control; *P<0.05 when compared with diabetic control.

Table -7: Change in body weight

Group	Weight in grams	
	0 day	**14th day**
3a	210.09±5.42	214.54±2.75
3b	218.13±5.72	219.75±4.82
3c	228.52±2.75	227.67±4.83
3d	237.02±3.82	239.74±5.23
3e	228.92±4.73	232.20±3.43
Standard	228.72±4.78	131.49±5.09
Diabetic control	215.25±3.98	205.49±3.62
Control	231.31±3.61	245.85±4.86

DISCUSSION

Different derivatives of 2-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one (3a-3e) were synthesized. The above synthesised compounds were screened for antidiabetic activity for alloxan induced-diabetes model. The effect of oral administration of synthetic derivatives are shown. Along with glucose other parameters including estimation of triglyerides, SGOT , SGPT , body weight were also observed.

In animals treated with alloxan a significant increase in the serum glucose, triglyerides, SGOT, SGPT levels and body weight was observed on the 7^{th},14^{th} day when compared to the normal group (G-8).

On administration of the standard drug glimepride (40 mg/kg) showed a significant decrease on described parameters on 7^{th},14^{th} day when compared to the diabetic control group (G-7).

Experimental studies revels that synthesized targeted compounds produced a significant decrease in blood glucose levels, because our titled compounds are framed with nuclei thiadiazole & thiazolidinone which have a tendency to reduce the type 2 type of diabeties.

Among the synthesised derivatives all compounds shows a significant hypoglycemic effect. In that ,3a derivative shows near action to the standard in serum glucose levels ie., compound with electron withdrawing group nor electron donating group makes the ring with prompt aromatic to exhibit action.

In serum triglycerides, among the (3a-3e), 3d ie., 3-OH derivative electron donating group at 3rd position on the ring exhibit significant action in the relation to standard.

Significantly ,3d (3-OH) derivative decreases the SGOT levels & 3e {$N(CH_3)_2$} derivative decreases the levels of SGPT ie., varying in functional group with also variance in position shows marked difference on estimated parameters, mentioned in our thesis.

In SGOT &SGPT levels, compound with electron donating groups on aromatic ring exhibit greater action than the others. Electron donating groups on ring are said to be activating, because they increase the rate of 2nd substitution. So that it is greater than that of normal benzaldehyde.

Here , body weight is another parameter need to consider. Generally on induction of alloxan resuls in increase in body weight on treatment with standard glimepride & test derivatives decrease the body weight. 3a derivative exhibits action near to the standard.

CONCLUSION

Our study reports the successful synthesis of 2-phenyl-3-(5-phenyl-1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-one (3a-3e) title compounds in good yields and showed marked antidiabetic activity (type -2) evaluated by the alloxan-induced diabetes in rat model. At last we observed the capitalization of antidiabetic activity, on inclusion of both thiadiazole and thiazolidinone nuclei in same structure. Thiadiazole acts by enhancing the activity of Glucokinase enzyme. Action of thiazolidinone is through inhibition of Aldose Reductase enzyme. Aldose reductase is a rate limiting enzyme in the polyol pathway associated with the conversion of glucose to sorbitol. Diabetic complications such as cataracts, nephropathy, and slowing of nerve conduction can be amliorted wih the use of these aldose reductase inhibitors. Diabetic complications such as cataracts, nephropathy, and slowing of nerve conduction can be amliorted with the use of these aldose reductase inhibitors. Our synthesized compounds believe to exerts its antidiabetic effect by one or both of the above mechanism. The investigation of activity reveled that 3a,3d,3e compounds showed good levels of antidiabetic activity.

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